Section 2:

Vector Management

UNDERSTANDING THE DYNAMICS OF NEONICOTINOID INSECTICIDAL ACTIVITY AGAINST THE GLASSY-WINGED SHARPSHOOTER: DEVELOPMENT OF TARGET THRESHOLDS IN GRAPEVINES

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ABSTRACT
The impact of systemic treatments of dinotefuran on the adult and egg stages of the glassy-winged sharpshooter (GWSS) is being evaluated using greenhouse and laboratory scale bioassays. One reason for the use of systemic treatments is that they exploit the xylophagous feeding behavior of the GWSS adult and immature stages. Our current data show that these treatments have an additional contact activity on emerging first instars before they begin feeding. In bioassays with adults exposed to grapevines treated systemically with dinotefuran, we quantified concentrations within the xylem by ELISA and related the concentrations to insect mortality. From these bioassays, we expect to generate a value that represents the effective concentration of dinotefuran needed to kill a GWSS adult feeding on a vine. This target threshold can then be used to guide growers in the selection of treatment rates, and as an indicator of the efficacy of treatments and the level of protection their vines are receiving.

LAYPERSON SUMMARY
The systemic neonicotinoids imidacloprid and dinotefuran are effective insecticides that growers can use for long-term management of glassy-winged sharpshooter (GWSS) populations. Because of the contrasting chemical properties of these insecticides, growers can now choose the most suitable product to meet their pest management needs. One of the interesting observations from this study has been that the concentrations of insecticide present within the xylem can be managed by choosing the appropriate application rate. This is a very powerful tool that can be used to optimize insecticide applications and manage insecticide use more effectively. In this study, we are determining the concentration of dinotefuran that is needed within the xylem of plants to kill a feeding GWSS. We have already demonstrated that dinotefuran is toxic to the GWSS adults, and we have also shown that nymphs emerging from an egg mass are susceptible to the contact activity of the insecticide before they commence feeding.

INTRODUCTION
Our research program focuses on the use of chemical insecticides for the management of glassy-winged sharpshooter (GWSS). We are dedicated to formulating safe and effective treatment programs for California growers, given the almost complete reliance by the grape industry on this method of control. We have conducted extensive trials in Coachella, Napa and Temecula valley vineyards to evaluate the uptake and persistence of three neonicotinoids – imidacloprid, thiamethoxam, and dinotefuran – under the diverse range of climatic, soil, and agronomic conditions associated with these regions. We have an understanding about how the different chemical properties, particularly water solubility, of these neonicotinoids can be exploited to achieve optimum uptake into vines, and we have developed sensitive techniques that allow us to monitor the levels of insecticide present within the vines. To exploit this knowledge further for the benefit of California grape production, we need to ensure that the concentrations of insecticide present within the vines are reaching levels that are effective at rapidly killing GWSS before they can infect vines with Pierce's disease (PD). We also need to understand whether there is a sub-lethal impact of these insecticides on GWSS, since anti-feedant activity may not necessarily eliminate the threat that an infective sharpshooter poses to a vine. Our past and current research projects have established the threshold levels of imidacloprid needed to kill a GWSS at 10 ng/ml xylem fluid, and optimized treatment regimes for growers that will ensure these thresholds are attained following applications via different irrigation methods (drip, sprinkler). In 2007, a new systemic neonicotinoid, Venom (active ingredient dinotefuran), received full registration for use on grapes. Our work in this area has demonstrated the excellent uptake of these new insecticides following systemic application to vines (Toscano et al., 2007). This is good news for vineyard operators who have experienced problems with imidacloprid. Imidacloprid has been the predominant neonicotinoid in use in vineyards, but our research has shown that its uptake and persistence within vines varies dramatically between regions (Coachella Valley, Napa Valley, Temecula Valley). Despite its apparent poor uptake, growers continue to rely on imidacloprid in many areas. The perception is that the insecticide will work well in all areas given its successful implementation in Temecula vineyards (Byrne and Toscano, 2006). Dinotefuran offers a potential solution to overcoming the problems encountered with imidacloprid use – its rate of uptake is faster and it can reach higher concentrations at peak uptake than imidacloprid under the more challenging situations. It also exhibits favorable persistence. Having established that the uptake and persistence of dinotefuran is superior to imidacloprid in terms of insecticidal titers reached in the xylem, it is important to ensure that the levels attained in the xylem are active against sharpshooters. Comparative data on the efficacies of systemic dinotefuran against GWSS are not available.

OBJECTIVE

1. Determine target thresholds for systemic neonicotinoids against GWSS in grapevines.

RESULTS AND DISCUSSION

The concentrations of dinotefuran in cotton plants used for bioassays of adult GWSS can be effectively controlled (**Figure 1**). In three independent experiments, the concentrations of dinotefuran in extracts of xylem fluid were consistent with the application rates used. After dilution of samples to eliminate matrix effects, the lower limit of detection of the ELISA was 30 ppb dinotefuran. This result shows that there is potential to control the levels of insecticide present in plants, provided there is a good understanding of the environment (soil type, irrigation, etc) under which the insecticide is being used.

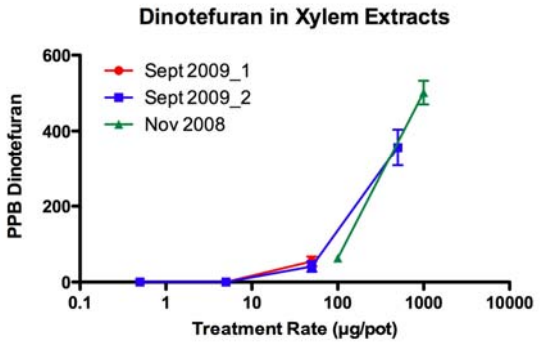

Figure 1. Concentrations of dinotefuran in xylem fluid sampled from cotton plants. Plants were treated with Venom 70 SG insecticide at different rates, and the xylem fluid extracted by pressure bomb at 5 days post-treatment when GWSS bioassays were completed. Not all concentrations were repeated for each bioassay.

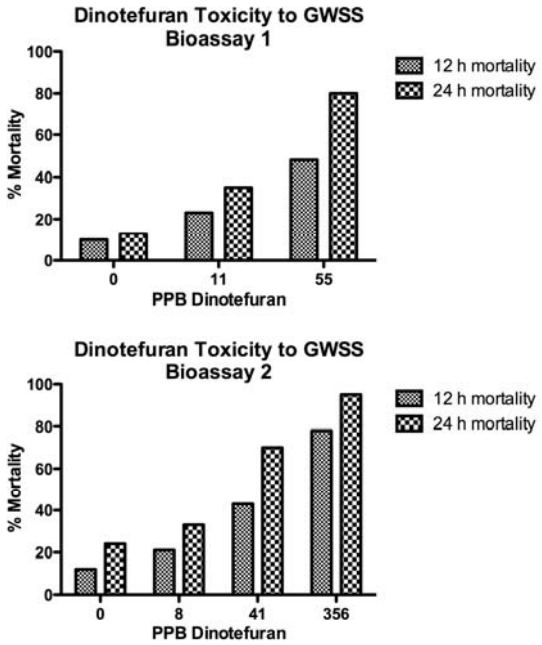

Figure 2. Toxicity of dinotefuran to GWSS adults. Mortality was assessed at 12 and 24 hours after the insects were confined on the treated plants. Data are from 2 independent bioassays.

The results of two independent bioassays are shown in **Figure 2**. Adult GWSS were caged on treated plants and mortality was assessed at 12 and 24 hours. In both bioassays, the control mortality was high. GWSS adults are difficult to work with because of the high control mortality, so it will be necessary to increase the number of replicates in subsequent bioassays in order to minimize this effect. Nevertheless, the results indicate the toxic nature of dinotefuran, with a clear dose response. The results from both bioassays were very consistent, indicating a robust bioassay system.

In the second set of experiments, we evaluated the effect of dinotefuran against the eggs of the GWSS. Adult GWSS were confined in cages with cotton, which is an excellent host for GWSS oviposition. Leaves with egg masses (not older than 24 hours) were cut from the plants and the petioles inserted into vials containing a range of insecticide solutions. The uptake of insecticide into each leaf was allowed to proceed for 24 hours and the leaves are then transferred to leaf boxes. The leaf boxes were maintained under lights until the normal period of embryonic development was completed. Mortality was assessed at the time of emergence of the first instar.

As with imidacloprid, the nymphs developed fully within the egg mass and only succumbed to the effects of contact with dinotefuran during emergence. In contrast to our previous data for imidacloprid, where we observed an LC_{50} of 39 ng/cm^2 leaf, the indications from our current data set show that dinotefuran is slightly more toxic to the first instars than imidacloprid (**Figure 3**). Also, the slope of the dose-response curve is extremely steep, as was observed for imidacloprid.

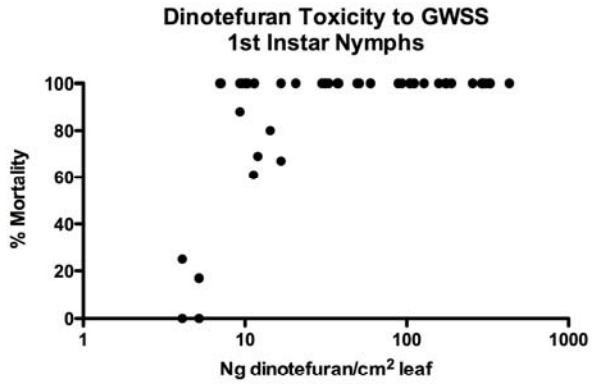

Figure 3. Toxicity of dinotefuran to emerging 1st instar GWSS. The petioles of leaves containing egg masses were placed in vials containing different concentrations of insecticide for 24 h systemic uptake. Leaves were then transferred to leaf boxes where the egg masses were allowed to continue their development. The survivorship of nymphs was determined for 2 days after emergence.

CONCLUSION

At current label recommendations, the rate of uptake of dinotefuran into grapevines is faster than imidacloprid and concentrations of dinotefuran at peak uptake are higher (Toscano et al., 2007). These two properties make dinotefuran a strong candidate for inclusion in a sharpshooter management strategy, provided that effective concentrations are reached within the xylem. Our bioassay data are inconclusive at this point due to high control mortality, which is making the true insecticidal effects difficult to ascertain. It will be important to minimize the impact of control mortality in order to derive an effective target concentration for dinotefuran against adult GWSS, so further bioassays are needed. Dinotefuran is highly toxic to emerging first instars, and our data suggest that the insecticide is slightly more toxic than imidacloprid. As with imidacloprid, the toxic effect is not manifested until the nymphs emerge from the egg mass, suggesting that dinotefuran and imidacloprid act as contact insecticides.

The systemic neonicotinoids imidacloprid and dinotefuran are effective insecticides that growers can use for long-term management of GWSS populations. Because of the contrasting chemical properties of these insecticides, growers can now choose the most suitable product to meet their pest management needs. One of the interesting observations from this study has been that the concentrations of insecticide present within the xylem can be managed by choosing the appropriate application rate. This is a very powerful tool that could be used to optimize insecticide applications and manage insecticide use more effectively.

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FUNDING AGENCIES

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DEVELOPMENT OF EFFECTIVE MONITORING TECHNIQUES FOR SHARPSHOOTERS AND THEIR PARASITOIDS

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ABSTRACT
This project relates to ongoing efforts of the Pierce's Disease Control Program to assess the efficacy of the sharpshooter egg parasitoid biocontrol program. *Gonatocerus morgani*, *G. morrilli*, and *G. triguttatus* have been reared and released by the program at sites throughout southern California and the southern Central Valley since 2000. California Department of Food and Agricultiure (CDFA) reports, the most recent in 2007, have demonstrated the effectiveness of these efforts. However, current methods to assess released species populations, the extent of parasitism by native competitors, and host preferences of the parasitoids involved are limited. D. Cooksey conducts research in comparative and functional genomics of *Xylella fastidiosa (Xf)* to identify key virulence factors through construction of specific mutations in the bacterial genome. To facilitate this work, his laboratory has developed a multiplex PCR system for the simultaneous identification of *Xf* strains (Hernandez-Martinez *et al.*, 2006). D. Morgan, Supervisor of the release program, is thoroughly familiar with the biology, ecology, systematics, and identification of the host and parasitoid species targeted in the proposed study. The development of the proposed multiplex PCR system will greatly enhance the data acquisition of the CDFA parasitoid release biocontrol program.

LAYPERSON SUMMARY
The suppression of glassy-winged sharpshooter (GWSS) populations is accomplished in part by biological control agents. An accurate and rapid method for identification of the eggs of sharpshooter species, determining whether eggs are parasitized, and by which parasitoid species, is essential for estimating success. Current methods are flawed and expensive. Development of a single-step multiplex real-time PCR assay for sharpshooters and their parasitoids would allow for accurate reporting of GWSS occurrences and facilitate development of effective control agents.

OBJECTIVES
1. Develop primer pairs that can be used in a multiplex PCR system for each species of sharpshooter and parasitoid. Several genes have been partially sequenced for GWSS and smoketree sharpshooter and for a number of their parasitoids. These sequences will be analyzed for primer design.
2. Clone the target genes from those species of parasitoid for which there is no sequence data available. This will be accomplished through the use of published primers or the development of degenerate primers.
3. Determine the limits of detection of each species of sharpshooter and parasitoid. Based on other studies, we are confident we will be able to detect developing parasitoid embryos in sharpshooter eggs. We hope to be able to determine both the host and parasitoid species from sharpshooter egg cases from which the parasitoids have eclosed by amplifying the layer of cells which remain in the parasitoid egg (Oda and Akiyama-Oda, 2008).

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FUNDING AGENCIES
Funding for this project will be provided by the University of California Pierce's Disease Research Grants Program.

RNA-INTERFERENCE AND CONTROL OF THE GLASSY-WINGED SHARPSHOOTER AND OTHER LEAFHOPPER VECTORS OF *XYLELLA FASTIDIOSA*

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ABSTRACT
This report presents the progress obtained in the development and application of an RNA interference (RNAi) based system aimed to target genes of the vector of *Xylella fastidiosa(Xf)*, the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*). After demonstrating that RNAi induction in GWSS cells and insects is achievable, in the year 2008-2009 we started screening a large pool of candidate genes to find the best target to control the survival of the insect vector. These data will be used to develop transgenic plants expressing dsRNAs of the target genes in their xylem tissues via EgCAD2, a xylem-specific promoter. Transgenic plants will be evaluated for their ability to induce RNAi effects on GWSS and other sharpshooter vectors of *Xf*.

LAYPERSON SUMMARY
This work presents fundamental efforts towards long term application of using RNA interference, RNAi, to help combat a plant disease of great economic importance. The disease, Pierce's disease (PD) of grapevines, is a significant threat to grape production in California and other parts of the U.S., and the causal agent of the disease, *Xylella fastidiosa (Xf)*, a xylem-limited bacterium, also causes several other extremely important plant diseases worldwide. Our effort here does not directly target *Xf*, but instead targets one of its most significant insect vectors, the glassy-winged sharpshooter (GWSS), and we combine the use of an *in vivo* system (GWSS whole insects) with an *in vitro* GWSS cell based system and demonstrate genetic and phenotypic RNAi effects. RNAi is an extremely important and broadly studied area in contemporary biology, and terms such as "magic bullet" for human medicine, and "genetic insecticide" for targeting insects have been used in the literature. Our work represents the first demonstrated RNAi effort in GWSS and our data will help to expand the possibilities to study plant-associated insects and at the same time to target the sharpshooter vectors of *Xf*, the causal agent of PD.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is among the most robust and thus most threatening vectors of *Xylella fastidiosa (Xf)*, the bacterium that causes Pierce's disease (PD) (Davis, Purcell et al. 1982), a devastating disease occurring in wine grapes from California to Texas to Florida (Myers, Sutton et al. 2007). New strategies that will lead to environmentally sound approaches to control GWSS and other insect vectors are needed. RNA interference (RNAi) has been suggested as a strategy to develop "insect-proof plants" (Gordon and Waterhouse 2007) and even referred to as a "genetic insecticide" (Scharf 2008). RNAi is a eukaryotic gene regulation/defense mechanism in which small RNA segments, small interfering RNAs (siRNAs) (21-25 nt), generated by processing of dsRNA molecules often of viral origin, specifically down-regulate complementary RNA sequences (Meister and Tuschl 2004). Recent efforts demonstrate that RNAi is inducible in many insects. Intrathoracic injection of dsRNAs has been shown to be the most effective way to induce RNAi in whole insects of many species including *Anopheles gambiae* (Blandin, Moita et al. 2002; Blair, Sanchez-Vargas et al. 2006), *Blattella germanica* (Ciudad, Piulachs et al. 2006), *Drosophila melanogaster* (Dzitoyeva, Dimitrijevic et al. 2001), *Spodoptera litura* (Rajagopal, Sivakumar et al. 2002), *Culex pipiens* (Sim and Denlinger 2009), *Lutzomyia longipalpis* (Mauricio R.V. Sant'Anna), *Cecropia pupae* (Bettencourt, Terenius et al. 2002), *Acyrthosiphon pisum* (Mutti, Louis et al.), *Rhodnius prolixus* (Araujo, Soares et al. 2009), *Aedes aegypti* (Cooper, Chamberlain et al. 2009), *Bemisia tabaci* (Murad Ghanim), *Dermacentor variabilis* (Mitchell Iii, Ross et al. 2007) and *Tribolium castaneum* (Arakane, Dixit et al.). Oral induction has also been demonstrated in several of these same species. Our effort demonstrates for the first time that RNAi activity can be induced in a leafhopper species, but also is inducible in GWSS cell lines. In the long term, RNAi can be used as an effective fundamental tool to better understand the dynamics of plant: pathogen: vector interactions as well as GWSS physiology and of course we hope as a strategy to complement overall efforts for PD control.

OBJECTIVES

The specific objectives of our effort are:

1. To identify RNAi-inducers capable of killing or reducing the survival and/or fecundity of GWSS and other sharpshooter vectors of *Xf*.
2. To generate transgenic plants capable of expressing GWSS deleterious interfering RNA molecules within their xylem.
3. To evaluate transgenic plants for their ability to induce RNAi effects vs. GWSS and other sharpshooter vectors of *Xf*.

RESULTS AND DISCUSSION

RNAi in GWSS cells and insects. Initially, we used 14 GWSS GenBank cDNA sequences corresponding to known proteins in order to synthesize RNAi inducer molecules, dsRNAs. We then tested whether RNAi was inducible in GWSS cells and insects, and we were able to show that RNAi activity is inducible in GWSS. Sets of dsRNA molecules were delivered to GWSS cells via lipid-based transfection, and to GWSS nymphs via intrathoracic injection or by feeding on cuttings immersed in a solution containing dsRNAs. Real time RT-PCR, semi quantitative RT-PCR, Northern blot of small and large RNA fractions showed that RNAi was achieved in cells and insects injected with dsRNA, where target mRNAs were partially degraded and specific siRNA, hallmarks of RNAi, were detected.

Because there are several potential sharpshooter vectors of *Xf*, the sequences isolated from GWSS were also amplified from the blue-green sharpshooter (BGSS; *Graphocephala atropunctata*) and from the green sharpshooter (*Draeculacephala minerva*) and cloned. This demonstrated a high degree of sequence conservation among these distinct sharpshooters, and the resulting sequences could be used to develop a general RNAi strategy to control multiple *Xf* vectors.

The above results showed anticipated reductions in target mRNAs. Therefore we evaluated if corresponding encoded proteins were reduced and if visible phenotypic results were induced. Western Blot analysis also showed a reduction of actin protein in GWSS nymphs injected with actin dsRNA (**Figure 1**). In addition, some of the injected nymphs did not complete ecdysis, demonstrating a striking phenotypic effect in whole insects vs. those injected with control gfp dsRNAs (**Figure 2**). A visible phenotypic effect was also obtained in GWSS cells transfected with actin dsRNA, where aberrations of the actin filaments occurred starting 72 hours post transfection. (**Figure 3**).

Because our results so far were dependent on a limited number of GWSS sequences so far available in Genbank, we analyzed three EST libraries deposited in GenBank. Twenty thousand thirty (20,030) EST sequences were analyzed using the Arthropod EST analysis pipeline at Kansas State University. One thousand nine hundred seventeen (1917) contigs were assembled and 6561 input reads were retained. The average length of the assembled contigs was 570 bp. NCB BLASTX was used to find sequence similarities in GenBank for the assembled contigs and singletons. One thousand seventy three (1073) contigs and 2057 singlets returned significant hits from GenBank, for a total of more than 3100 sequences. As expected, the great majority of these sequences correspond to structural and housekeeping genes, but a great number correspond to genes of potential interest as RNAi targets, including genes for cuticle formation, larval development, juvenile hormones, central nervous system development, eye morphogenesis and development, lipid and carbohydrate metabolism expressed in gut tissues and genes expressed specifically in salivary glands. Experiments are underway to begin assessing these potential RNAi targets.

Xylem specific promoter cloning.

The specific xylem promoter EgCAD2 was cloned from *Eucalyptus gunii*. The sequence was fused to the GUS reporter gene in the binary PCB 301 vector. Then, GUS expression driven by the xylem specific promoter was accessed in a transient *Agrobacterium tumefaciens* assay in *N. benthamiana* plants. Upon staining for GUS activity, results showed that blue product was restricted to the main vascular tissues. This gives confidence in this promoter, which will now be used to attempt to express specific interfering RNAs in the xylem of transgenic plants. Choosing which plant to use initially is difficult. However, citrus has been implicated as an important GWSS host plant in southern California, and Carrizo citrange is one of the plants easily transformed and manipulated at UC Davis. It also is a host of GWSS in our studies (**Figure 4**), thus it will allow us to rapidly test our hypothesis for xylem delivery of RNAi molecules.

CONCLUSIONS

Xf is an important bacterial pathogen of economically important crops such as grape, but also citrus and almond. The ability to minimize the economic impact of this bacterium depends on the presence and abundance of its biological vectors and GWSS is the most effective vector of *Xf* transmission in some agricultural areas. RNAi-based efforts directed toward the control of insect plant pests are now becoming more feasible, and RNAi for insects as GWSS has great potential application.

The results presented here show that RNAi can be induced both *in vitro* (GWSS -Z15 derived cell line) and *in vivo* in GWSS nymphs. We showed that GWSS -Z15 cells can be used to screen candidate gene silencing targets, and that since RNAi is active in cells, it could also be used to study GWSS gene function via mRNA knockdown. The mRNAs targeted for RNAi in this study were chosen from a limited number of sequences currently available for GWSS, but the same approach can be applied to the other genes identified in the analysis of the GenBank GWSS EST libraries. More notably, the employment of RNA silencing in whole GWSS insects could offer help towards a potential solution for control of the vector. Future work

includes the screening of more RNAi targets, the production of transgenic plants expressing dsRNAs in their xylem and the study of GWSS insects grown on the transgenic plants.

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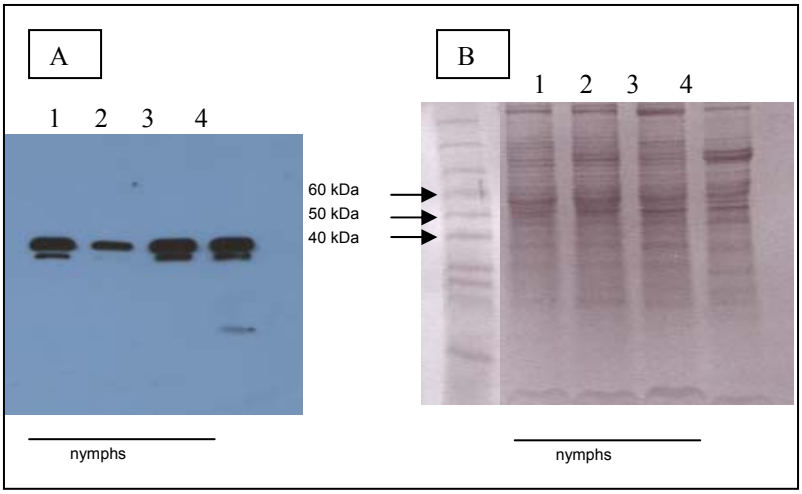

Figure 1. GWSS nymph injected with actin dsRNA shows decreased actin protein level. Fifteen third and fourth instar GWSS nymphs were injected with 1µg actin dsRNA in 1µl volume, with 1 µg GFP dsRNA or with 1 µl injection buffer and left on basil plants for five days. Then, proteins were extracted from three living and one dead insect and subjeted to Western blot analysis, using actin antibodies specific for *Drosophila melanogaster* . Results show a decrease in actin protein in the nymph injected with actin dsRNA and alive five days post injection (gel lane 2 panel A), compared to the other insects (gel lanes 1, 3 and 4 in panel A). Coomassie staining shows equal amounts of proteins loaded for each sample (panel B), 15 µg total proteins were loaded in each lane. Treatments: Lane 1: Actin dsRNA injected nymph, collected dead one day after injection. Lane 2: Actin dsRNA injected nymph collected dead five days after injection. Lane 3: GFP dsRNA injected nymph collected dead five day after injection. Lane 4: Uninjected adult.

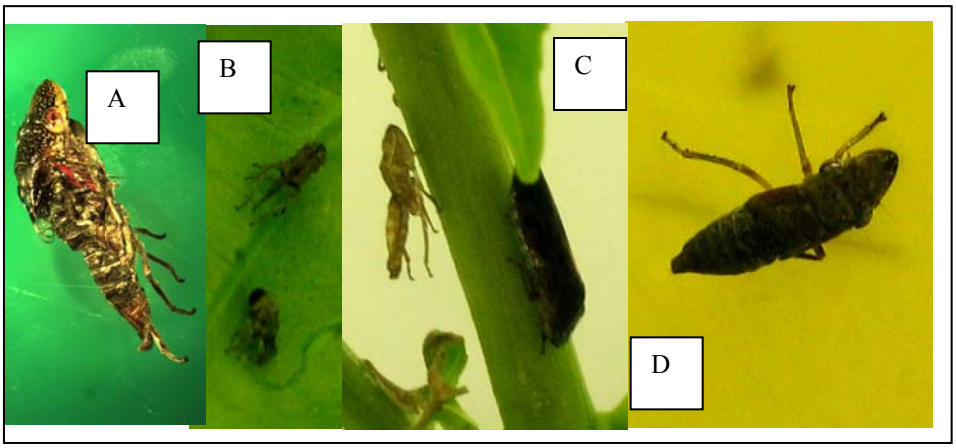

Figure 2. GWSS nymphs injected with actin dsRNA died during molting. Fifteen third and fourth instar GWSS nymphs were injected with 1ug actin dsRNA in 1ul, or with 1 ul injection buffer and left on basil plants for five days. During this period, two of the actin dsRNA injected insects couldn't complete molting and died. In panel A, one of the nymphs with incomplete molting is shown. In other panels the presence of exoskeletons on a basil leaf indicate the completion of molting in the observed group of nymphs (picture B). Shot of an exoskelton close to an adult that succesfully completed molting (picture C). Injected nymph showing a normal phenotype (picture D). Experiment was repeated three times with similar outcome.

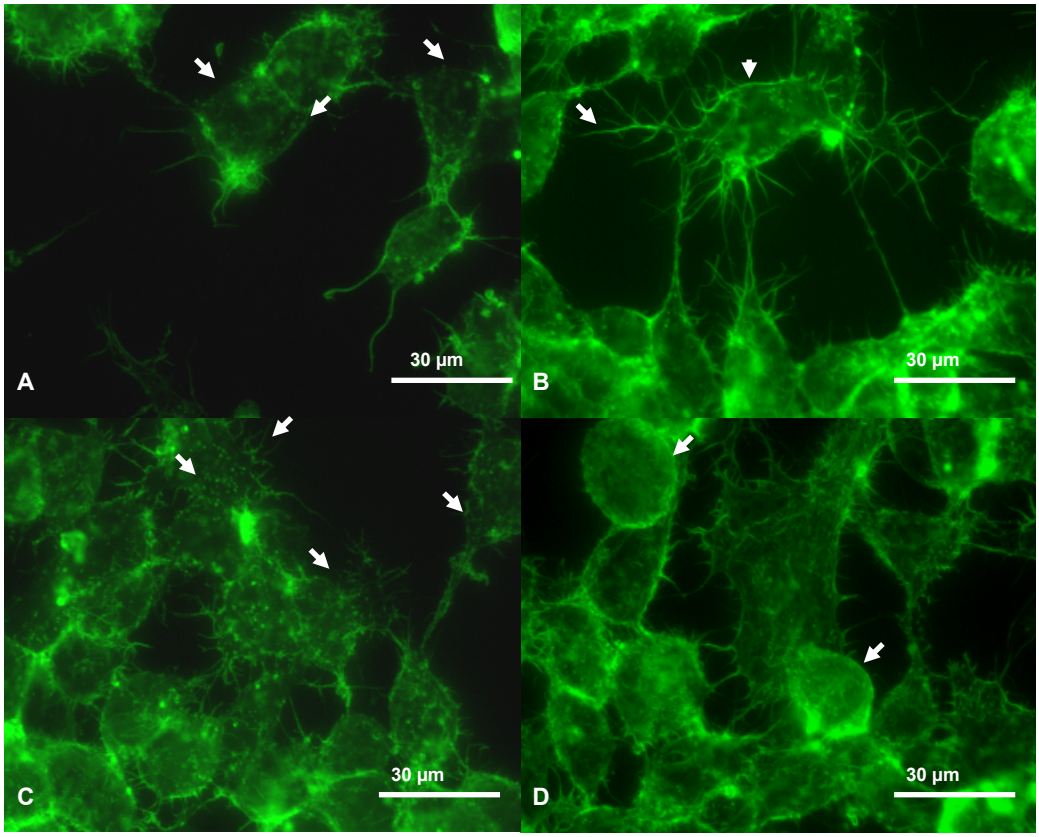

Figure 3. Actin representative morphology in GWSS -Z15 cells after transfection with actin dsRNA. Cells were transfected with 2 µg of actin dsRNA (A and C), or GFP dsRNA (B and D) and harvested 72 hpt. Actin filaments in the cell membrane and cytoplasmic area were largely disturbed (arrows in A and C). (A) GWSS cells showing partial disruption of the actin organization at the cell plasma membrane. Some filaments began to break and the cells failed to branch out. (B) GWSS cells showing no changes in actin filament distribution and polymerization. Healthy isolated cells were connected through a densely branched actin filament network. (C) GWSS cells showing severe disruption of actin filaments. The short fragments of actin filaments were scattered throughout the cytoplasm. Some actin fragments tended to aggregate into clusters below the plasma membrane and obvious twisted actin cables could be observed. (D) Actin filaments were found primarily in the cell cytoplasm as a continuous and organized net in the control cells. All observations were at 72 hours post treatment.

Figure 4. GWSS adults and nymphs feeding on Carrizo seedlings. Two GWSS adults and two nymphs were introduced in cylindrical plastic cages containing one month old Carrizo seedlings and left feeding for one week. After this period of time, all insects were alive and were feeding on the seedlings. Plants did not show any damage caused by the GWSS feeding.

IMPROVED DETECTION, MONITORING, AND MANAGEMENT OF THE GLASSY-WINGED SHARPSHOOTER

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Reporting Period: The results reported here are from work conducted July 2007 to October 2009.

ABSTRACT
Efficient and precise methods for detection of new colony infestations and for monitoring glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis* (Germar)) populations are lacking. This proposal addressed detection and monitoring methods and accompanying leafhopper behavior toward improved management of GWSS.

LAYPERSON SUMMARY
Management of the vector glassy-winged sharpshooter (GWSS) and Pierce's disease (PD) is contingent on the availability of efficient field-sampling methods. This proposal aimed to improve upon the current monitoring methods. GWSS behavior in response to various types of traps in combination with host plants and other factors with potential to increase trap efficiency were investigated. Current trapping relies on use of the yellow Seabright trap which is flat with two sides covered with stickem to capture the insects. The trap attracts in two directions and has a total yellow attraction area of 653.8 cm^2 and a trapping surface area (stickem covered) of 409.4 cm^2. We have found that a yellow cylinder (tube) trap 7.6 × 30.5 cm (3 × 12 in, 730 cm^2 area) that samples in all directions (360^0) usually improves trap capture rate by two-four times for males and somewhat less for females. We have used Glidden Alkyd Industrial Enamel 4540, Safety Yellow, as the standard color and Tangletrap™ (Gemplers.com) as the standard sticky substance. Trap capture efficiency is inversely proportional to the distance from a host plant. GWSS respond to other leafhoppers when searching for and landing on a host plant and apparently in response to traps. Adding a leafhopper or model of a leafhopper to a trap can increase trap catch by 20-50% under low vector populations. Trap capture efficiency does not correlate well to leafhopper numbers found on host plants with the exception of within large blocks of citrus (Castle and Naranjo 2008), and may be inversely related on host plants with high nutritional quality such as crape myrtle when the plant is at peak quality with respect to xylem nutrients.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) as a vector of *Xylella fastidiosa (Xf),* remains a threat to grapes, almonds, stone fruit and oleander and impacts citrus and nursery crops throughout much of California. It remains an important quarantine pest for the Napa and Sonoma Valleys and other critical uninfested locations. Due to the unique biology and behavior of GWSS which is driven by plant xylem chemistry and nutrition, conventional detection and monitoring approaches may not provide the necessary statistical precision needed by the regulatory and producer community for management decisions. This proposal addressed the detection and monitoring needs.

OBJECTIVES
Overall: To determine the most efficient and cost effective trapping system to detect and monitor GWSS population dynamics and the potential to manage GWSS populations.
1. Evaluate and summarize previous sampling and trapping efforts for GWSS.
2. Trap configuration and number: Determine the potential and optimize the number of traps that are most efficient and cost effective in detecting and estimating GWSS populations.
3. Determine the effects of host plants in combination with traps: Determine the potential and the optimization of a combination of GWSS host plants in sentinel plots to detect, estimate and manage GWSS population dynamics.

RESULTS AND DISCUSSION
A series of data are provided to indicate some of the approaches we have undertaken. In brief, we have looked at trap size, color, height, shape, orientation, background contrast, placement relative to vegetation, distance from vegetation and a number of other factors relative to GWSS behaviors with the objective of understanding and improving trap efficiency. We have also made some novel discoveries about GWSS behavior in response to congeners. We have used the commercially-available Glidden Alkyd Industrial Enamel 4540 Safety Yellow, as the standard color in all tests versus commercial traps, Tangletrap™ (www.Gemplers.com) as the standard sticky substance, and a standard height of 1 m from the ground for trap placement.

We have shown that GWSS capture rate may be increased by changing the trap configuration from a flat two-sided trap into a cylinder (tube) shape which apparently samples the entire surrounding 360^0. A comparison of safety yellow mailing tubes 5.1 cm width × 15.2 cm or 30.5 cm length, 7.62 cm width × 15.2 cm or 20.5 cm width, and 10.2 cm width × 15.2 cm length or 20.5 cm length indicated that total GWSS trap capture increased approximately 40-50% in response to each incremental

increase in trap size either in width or length. All tube sizes captured significantly more GWSS than the Pherocon AM trap used as the standard in these experiments. We choose a 7.62 cm × 15.2 cm as a standard size of the tube trap because it improves capture rate, is easy to work with and less expensive. In other experiments we tested the tube trap versus the Pherocon AM and the Seabright flat, 2-sided yellow traps as well as versions of the commercial traps configured into a cylinder shape with mixed results. The tube trap always captures numerically greater numbers of GWSS but it was usually only the male leafhoppers that were captured in significantly higher numbers. For example in one test the tube captured 125 (total) leafhoppers (93 males, significantly greater than other treatments, t-test, $P<0.001$) while 31 (24 males), 25 (17 males) and 37 (23 males) were captured by the Pherocon AM, Seabright and the Seabright cylinder, respectively. In another test, the Pherocon AM captured 10.5 ± 3.5 (mean ± SEM)/trap/sample period, Pherocon AM cylinder captured 12 ± 3.5 and the tube captured 21 ± 3.4. In another test we deployed 10 traps of the tube, the standard Seabright and two Seabright traps together formed into a cylinder with staples to provide the same surface area and relative profile as the tube trap. We placed the traps on one m high stakes in a RCB design in a block of large crape myrtle. The Seabright captured a total of 142 GWSS (122 males), the Seabright cylinder 295 (260 males) and the tube captured a total of 389 (337 males) over the duration of the test. All the trap types were significantly different ($P<0.0001$) using SAS PROC GENMOD and contrast tests to compare means. These results appear to indicate that the cylinder shape provides an advantage but we did not control for the difference in yellow hue inherent in the trap colors. Nevertheless, color does not appear to be the main factor because it is likely that the Seabright cylinder capture rate was in part lower due to our inability to make it completely smooth when we constructed it from two separate traps. However, the Seabright cylinder trap presented a larger target overall because we formed the trap such that the area covered with stickem was equal to that of the tube trap. All things being equal, the tube trap provided a significant increase in capture rate.

Another method to potentially increase trap capture rate and efficiency may be to increase the number of traps used together or to enhance the attraction of the main sticky trap with some additional visual cues. Size matters as explained above. The addition of a host plant clearly increases the number and/or the residence time of responding leafhoppers thereby bringing them in closer contact with the trap. This might function by increasing the active distance of the trap to the leafhoppers, thus in effect sampling more area around the trap. We tested this concept in several ways in several tests with both Seabright and tube traps as follows but found no significant increase in trap capture rate. We placed traps in the field using an randomized complete block (RCB) design with treatments of one, two and three traps together two m apart. We placed sticky traps ≤ 0.5 m away from another yellow tube without stickem that was 7.6 cm in width but 91 cm in length (bottom 30 cm next to trap and top 60 cm above the sticky trap). We also placed the larger yellow tube without stickem directly above the target sticky trap. These results appear to indicate that GWSS respond directly to traps and do not spend time in any behavior around traps once they respond such as moving down, repeated flying around into the trap, etc. that may be exploited. We also tested a treatment that placed the larger tube without stickem below the target trap (ground -one m) without significant improvement in trap catch.

GWSS respond strongly to host plant quality and change host plants often. Optimum trap placement relative to host plants was considered as one potential method to improve trap capture rates. Several tests were conducted to investigate GWSS response to traps relative to the presence of a host plant, host plant quality and the trap distance from a host plant. **Figure 1** shows the effect of host plant presence on trap catch using tube traps and a poor host peach vs a good host crape myrtle. Twenty-five container plants each of peach and crape myrtle with adjacent traps and 25 traps without plants were placed in the field in adjacent blocks in a five × five m grid. GWSS were recorded each day in the morning and afternoon by position either on the plant or trap. The treatments were re-randomized one time per week to remove any positional effects in the field plot. Clearly, the presence and quality of the host plant affected the number of GWSS trapped with the better host crape myrtle attracting more and inducing a higher trap capture rate than peach, the poor host plant, and the traps alone. In a different study we used tube traps in combination with an array of host plants in containers and compared trap capture between traps with plants within one m distance and traps without plants. Host plants used were apple, red oak, 'Tonto' crape myrtle, 'Flordaking', and 'Elberta' peach, redbud, 'Santa Rosa' plum and 'Bradford' pear. We used seven replicates of each treatment in a RCB design and conducted the test for 38 days. The trap alone captured 135 GWSS and all trap + plant treatments, except for 'Flordaking' peach and 'Santa Rosa' plum which captured less, numerically captured a higher number, on average 47% more, GWSS than the control. Apple, oak, redbud and Bradford pear traps captured statistically significantly higher GWSS than the control ($P<0.05$, LSD).

Along with plant quality differences, the distance a trap is placed from a plant may also be a factor potentially affecting GWSS trap capture rates. In another test we placed a seven × seven m grid of tube traps 10 m apart centered on a large planting of 'Natchez' crape myrtle of ca. two m in height (see other results from this test reported in Northfield et al. 2009). Response of GWSS to traps located at different distances from the crape myrtle plants are shown in **Figure 2**. A linear relationship inversely related to distance was significant at $P< 0.001$ with an $r^2 =0.58$. In another test we placed five replicates of tube traps and Seabright traps at one m and five m from large 'Natchez' crape myrtles. In this test GWSS response was: tube 8.7/trap period ± 0.86 (mean ± SEM) at 1 m, and 4.4 ± 0.6 at 5 m, and Seabright 5.2 ± 1.24 at one m and 4.6 ± 0.75 at 5 m. The tube at one m had significantly higher GWSS ($P < 0.0006$) using SAS Proc GENMOD and contrast statements. Again, the majority of leafhoppers captured were males. Finally, trap efficiency is directly related to how much surface area the trap actually samples termed here as the trap's "active distance". In the case of a cylinder this would be

circle of some size around the trap. This parameter is directly related to the size of the trap given that color and height are optimized. We attempted to determine the active distance of the tube trap by placing a set of five traps in a configuration where a center trap was surrounded by four other traps in a square around it. We varied the distance of the surrounding square of four traps by six, eight, 10 and 12 m. We also placed a single control trap 35 m away from the treatment trap sets. We used five replicates in a RCB design. Theoretically, the active distance is determined by comparing the treatment capture rates in the center trap to the control trap rates and by comparing the total capture rate between treatments. When the center and control rates are equal then the active distance of the trap is somewhere near this spacing because at lower distances the four companion traps interfere with the center trap capture rate which is lower than the control as a result. When the total capture rates per treatment decrease, the trap groupings change at that distance into independent traps rather than acting together (overlapping as in a single visual presentation) as they would at lower distances. Our results were unclear, however, we observed no differences between the center and control traps but total GWSS trap capture rate by treatment did increase linearly from six, eight and 10 m and then declined in the 12 m treatment. This suggests that the tube trap may have an active distance of approximately 10 m.

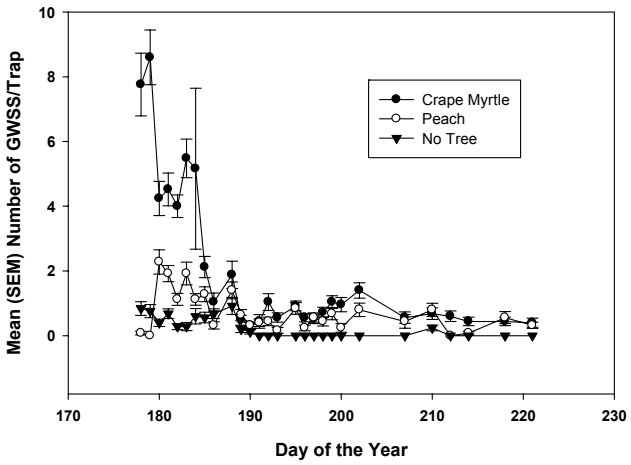

Figure 1. Relationship of GWSS trap capture rate to the presence and absence of host plants: peach and crape myrtle represent poor and good host plants, respectively. Traps used were standard tube traps.

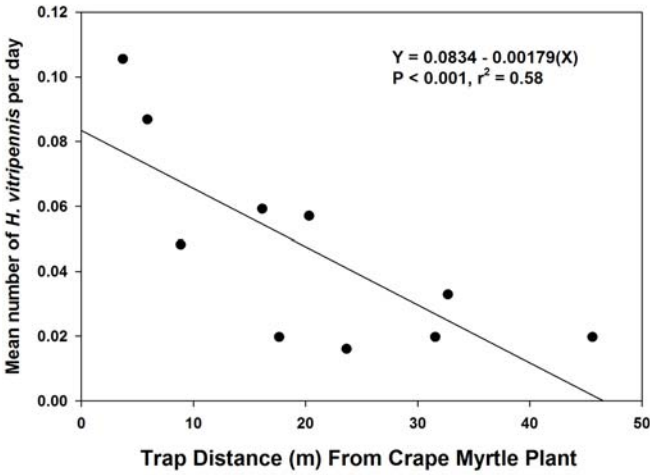

Figure 2. Response of GWSS to traps located at different distances from large 'Natchez' crape myrtle plants. Data points have been normalized by placing them in increments of 10 m centered on the values along the X axis.

The above results, observations of GWSS aggregation behavior in the field on host plants, in response to host plants and on traps led us to examine in depth the response of leafhoppers to traps and to other leafhoppers. The distribution of GWSS landing on traps was investigated with and without other GWSS present. **Figure 3** indicates the natural distribution of both sexes of GWSS on the Seabright trap and shows that GWSS tend to aggregate naturally in the lower right hand quadrant. That is in blocks three-five (left to right) of the Seabright trap. **Figure 4** shows how the GWSS responded to the Seabright trap when there was a dead GWSS carcass added to the center block on the trap. In this test we allowed only one leafhopper to respond each time to eliminate the confounding effect of previously trapped leafhoppers on the behavior of newly arriving leafhoppers. The presence of a leafhopper carcass shifted the distribution of the arriving leafhoppers to the center area of the trap with males landing at significantly higher numbers in blocks one and two from the center while females landed in higher numbers in blocks two and four. In another test, GWSS response to an unbaited control Seabright trap was compared to treatments of one, two or three GWSS carcasses or one, two or three black plastic models similar in size to GWSS added to the center boxes of one side of Seabright traps. Five replicates of each treatment were used in a RCB design. Responding GWSS were recorded by the block number away from the carcass where they landed as was the number of GWSS that landed on the unbaited side of the trap. Overall the baits increased trap catch by 55% on the baited side versus the unbaited side (data not shown). Other tests conducted included the addition of a small 15 cm long tree branch with GWSS carcasses on it to tube traps in parallel with the trap orientation and the addition of a similar small branch with carcasses attached to the tube trap in the middle and sticking out at a 45^0 angle away from the trap versus an unbaited control. Neither of these treatments provided significant increase in trap captures. Thus, the response to congeners appears to be a short range landing orientations. Nevertheless, we are pursuing this response to congener behavior by GWSS to further describe what is actually occurring and our results will be reported orally at the symposium.

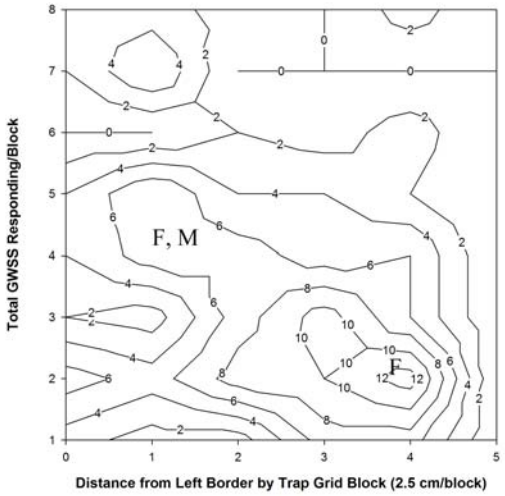

Figure 3. **GWSS landing distribution on a Seabright** trap without any other GWSS present. M is peak point of males and F indicates female peaks.

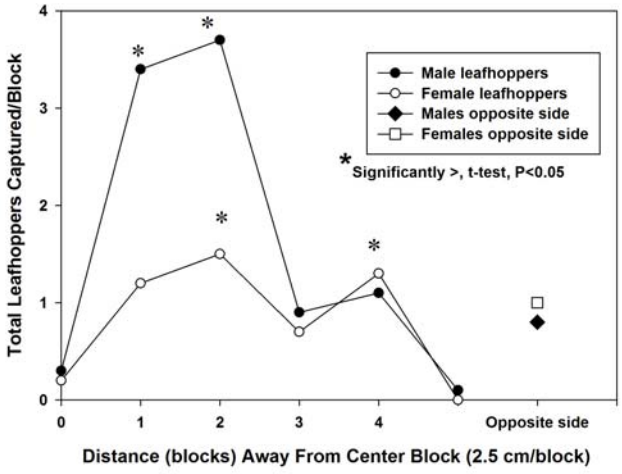

Figure 4. Response of GWSS to Seabright traps baited in the center block with a GWSS carcass.

CONCLUSIONS:

The experiments conducted in Florida under this grant were all completed in locations with relatively low GWSS populations. These low population conditions typify what would occur under a regulatory/quarantine function when the objective is to detect newly establishing populations when they first likely occur in very low numbers. The collective results of these studies suggest that the detection and monitoring efficiency of trapping of GWSS may be improved in a number of ways. Some are highly practical, whereas others would require a much different approach than the conventional deployment of traps haphazardly in some fashion in the field using a transect or a grid of traps. Use of traps for estimating populations within plants appears to be highly ineffective in most cases with the exception of crops in large acreage blocks where there is little immigration and emigration occurring in the sample location (Castle and Naranjo 2008, Northfield et al. 2009). Changing from the Seabright trap to a cylinder tube trap would improve detection levels but may be impractical relative to costs and logistics required shipping, moving and storage. Response by GWSS to congeners is novel and may have significant value. We are pursuing this aspect of GWSS behavior. The addition of a printed black silhouette GWSS model to the middle of the Seabright trap may improve its efficiency but this requires more testing under high populations. An increase in trap capture rate may accrue simply from the model's result of shifting the position of GWSS landings on the trap to nearer the center where they are surrounded by larger areas of sticky surface which may decrease their ability to escape the trap. Once leafhoppers arrive on the trap the model effect may lose value. However, from a regulatory perspective this change may be of value, but requires more tests. The attention to trap placement relative to host plant quality, distance from plants and trap size all can be considered for improving trap efficiency and the probability of early detection of low GWSS populations. It does not appear that GWSS exhibit any unusual and exploitable behaviors in either long or short range response to single or multiple trap configurations that may be exploited to improve trap capture rate.

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FUNDING AGENCIES

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RIVERSIDE COUNTY GLASSY-WINGED SHARPSHOOTER
AREA-WIDE MANAGEMENT PROGRAM IN THE COACHELLA AND TEMECULA VALLEYS

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ABSTRACT
Riverside County has two general areas where citrus groves interface with vineyards, the Coachella and Temecula Valleys. The Coachella Valley with 10,438 acres of table grapes in proximity to 12,000 acres of citrus and the Temecula Valley with 2,000 acres of wine grapes in proximity to 1,000 acres of citrus are vulnerable to Pierce's disease (PD). The grapes in the Coachella and Temecula areas of Riverside County are in jeopardy because of the glassy-winged sharpshooter (GWSS), the vector of the PD bacterium, build up in adjacent citrus groves. Citrus is an important year around reproductive host of GWSS in Riverside County, but also one that concentrates GWSS populations over the winter months during the time that grapes and many ornamental hosts are dormant. GWSS weekly monitoring in citrus in grapes began in March 2000 in Temecula Valley and 2003 in Coachella Valley by trapping and visual inspections. Temecula valley GWSS populations in 2008 reached levels not seen prior to the initiation of the area wide GWSS program in 2000. Coachella Valley GWSS populations have decreased dramatically since the treatment program was initiated in 2003.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) vectors a bacterium that causes Pierce's disease (PD). This insect and bacterium are a severe threat to California's 890,000 acres of vineyards and $30 billion dollar industry. An area-wide GWSS management program was initiated in Temecula in 2000 to prevent this vector's spread into other California grape growing regions. In Temecula Valley itself, the wine grape industry and its connecting tourist industry generate $100 million of revenue for the economy of the area. GWSS/PD caused a 40% vineyard loss and almost destroyed the connecting tourist industry. The area wide GWSS management program initiated in the spring of 2000 saved the industry from a 100% loss. Only a continuation of an area-wide GWSS management program will keep the vineyards viable in Temecula. The table grape industry in the Coachella Valley is represented by 10,465 acres of producing vines, which generate fresh market grapes valued at an average of $110 plus million annually. The GWSS was identified in the Coachella Valley in the early 1990's. Population increases of this insect in Coachella Valley in the last three years have increased the danger of PD occurrence in this area, as has occurred in similar situations in the Temecula and San Joaquin Valleys. In July 2002, the occurrence of *Xf,* the PD bacterium, was found in 13 vines from two adjacent vineyards in the southeastern part of Coachella Valley. With this discovery, and the increasing GWSS populations, there was and is a real need to continue an area-wide GWSS/PD management program. The GWSS area wide management program is needed to prevent an economic disaster to the work forces and connecting small businesses of Mecca, Thermal, Coachella, Indio, etc. that depend upon the vineyards for a big portion of their incomes. Only a continuation of an area wide GWSS/PD management program will keep the vineyards viable in Coachella. At present there are no apparent biological or climatological factors that will limit the spread of GWSS or PD. GWSS has the potential to develop high population densities in citrus. Insecticide treatments in citrus groves preceded and followed by trapping and visual inspections to determine the effectiveness of these treatments are needed to manage this devastating insect vector and bacterium. Approximately 1,600 acres of citrus in Riverside County were treated for the GWSS in April through September, 2009 between a cooperative agreement with USDA-APHIS and the Riverside Agricultural Commissioner's Office under the "Area-Wide Management of the Glassy-Winged Sharpshooter in the Coachella and Temecula Valleys".

OBJECTIVES
1. Delineate the areas to be targeted for follow-up treatments to suppress GWSS populations in the Temecula and Coachella Valleys for 2010.
2. Determine the impact of the GWSS area-wide treatments to suppress GWSS populations in citrus groves and adjacent vineyards.

METHODS, RESULTS AND CONCLUSIONS
The programs in Coachella and Temecula were dependent upon grower, pest management consultants, citrus and vineyard manager's participation. The areas encompass approximately 28,000 acres. Representatives of various agencies were involved in the program, they were as follows: USDA Agricultural Research Service, USDA Animal and Plant Health Inspection Service, California Department of Food and Agriculture, Riverside County Agricultural Commissioner's Office, University of California-Riverside, UC Cooperative Extension, and grower consultants. Representatives of these agencies meet to review the program. Newsletters are sent to growers, managers, wineries, and agencies with information on GWSS

populations and insecticide treatments via e-mail. The information from Temecula is sent weekly, while information from Coachella goes to the various parties monthly.

The GWSS/PD citrus groves and vineyards within the GWSS/PD management areas were monitored weekly to determine the need and effect of insecticide treatments on GWSS populations. In August, 2008, because of the lack of GWSS trap catches in Coachella valley, a bi-weekly schedule was initiated. Yellow sticky traps (7 x 9 inches) were used help determine GWSS population densities and dispersal/movement within groves and into vineyards (**Figures.1 & 2**). Approximately 1,400 GWSS yellow sticky traps are monitored in the Riverside county area wide program. Based on trap counts and visual inspection, approximately 1,000 acres of citrus were treated in Temecula valley for GWSS in 2009. In 2009, 600 acres of citrus were treated in Coachella Valley for GWSS area wide management. Because of high Temecula GWSS trap catches in the late summer and early autumn of 2008 and GWSS trap catches in January, 2009, imidacloprid (Admire Pro) applications in citrus were initiated in April, 2009 (**Figure 3**). Admire Pro was applied at the rate of 14 oz/acre. Of the 1,000 acres of treated citrus, 72 acres of organically farmed citrus were treated with Omni Oil 6E at the rate of 1%/acre and PyGanic (1.4% Pyrethrins) at 18 oz/acre. Because of the low residual of the organic insecticides the organic citrus was treated three times during the season. Omni Oil was applied in June on the citrus, followed by PyGanic treatments in July and September.

For a successful area-wide GWSS management program with large acreages of citrus, a management program has to be maintained. Organic insecticides are not as effective as the neonicotinoid insecticides such as imidacloprid for controlling GWSS. Therefore, organic insecticides will have to be applied more frequently than its synthetic counterpart. In our Riverside County GWSS area wide program organic citrus groves pose challenges to area-wide GWSS management programs.

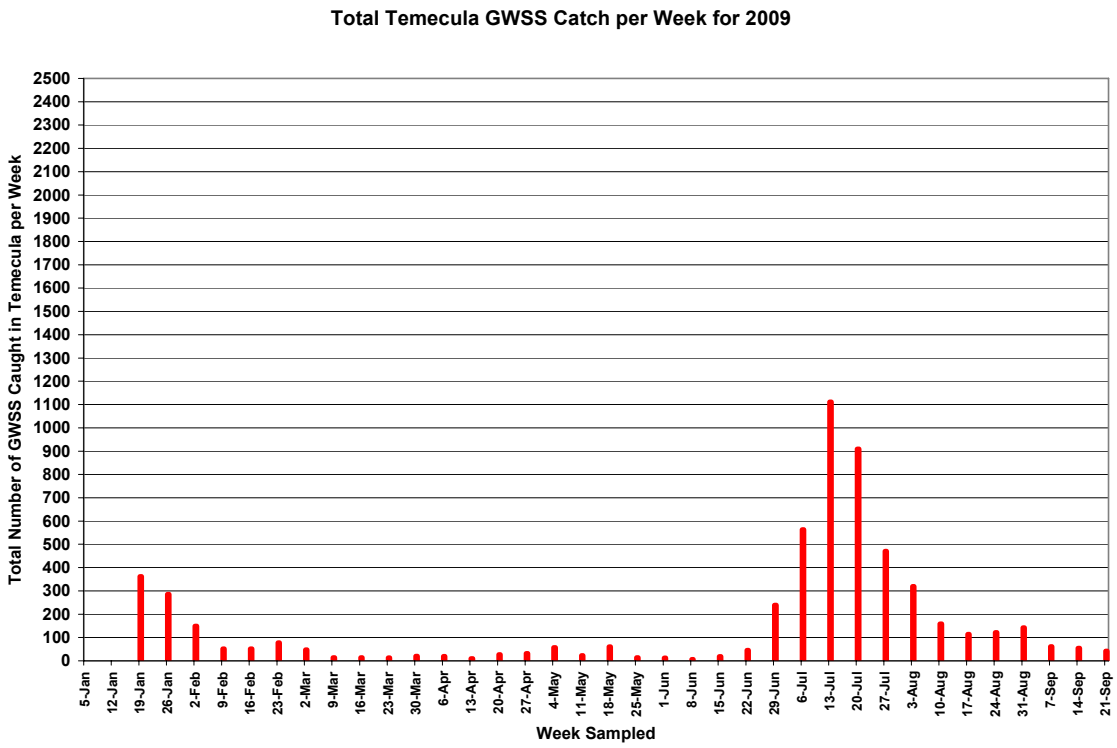

Figure 1. In 2009, high numbers of adult glassy-winged sharpshooters were caught on the yellow sticky traps in Temecula, with populations peaking in July reaching a total of approximately 1,100 trapped.

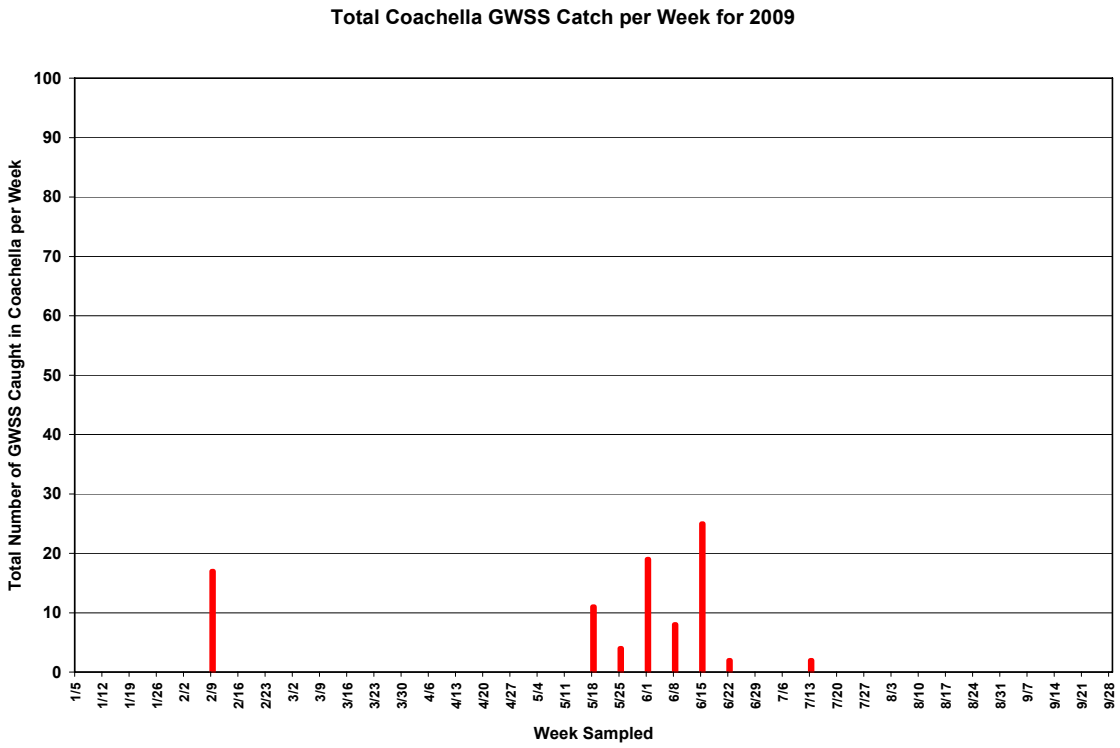

Figure 2. Glassy-winged sharpshooter populations in Coachella Valley peaked in June with a high of 25 trapped.

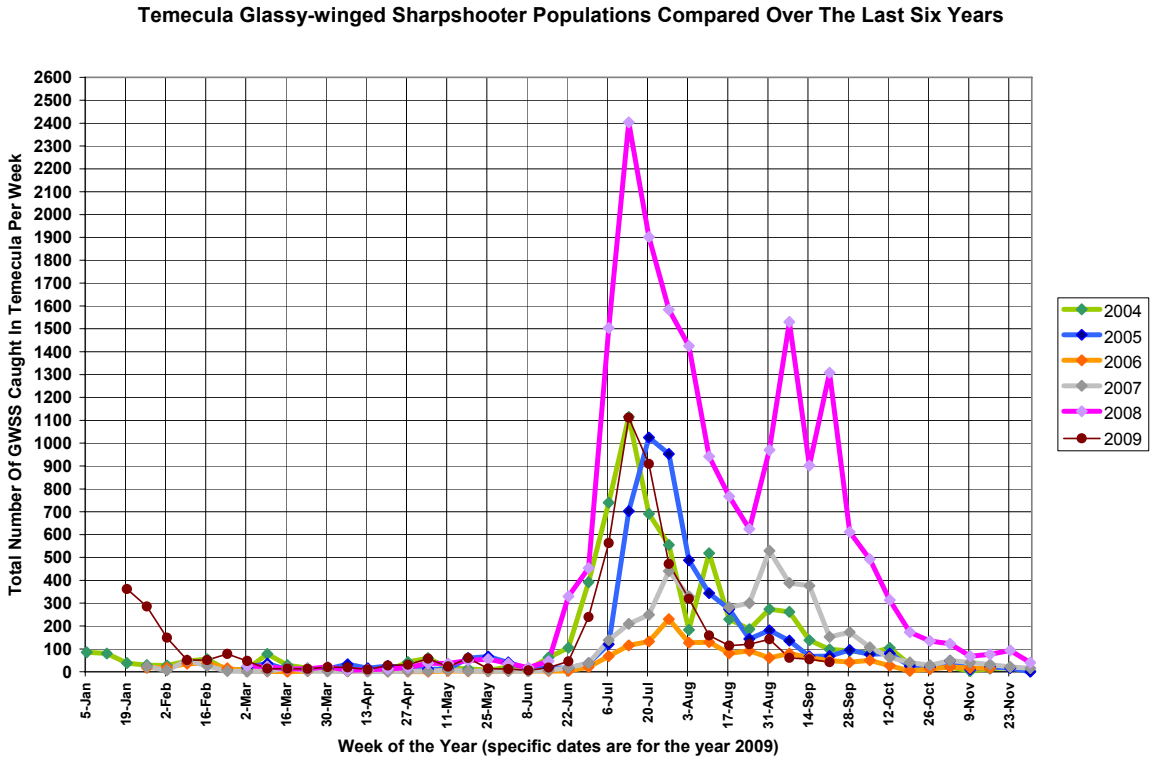

Figure 3. Glassy-winged Sharpshooter populations compared over the last six years

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